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(57) Abstract

The invention relates to novel methods and compositions for the detection of analytes using the nuclear reorganization energy, λ, of an electron transfer process.

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DETECTION OF ANALYTES USING REORGANIZATION ENERGY

This application is a continuing application of U.S.S.N. 08/873,977, filed June 12, 1997.

FIELD OF THE INVENTION

The invention relates to novel methods and compositions for the detection of analytes based on changes in the nuclear reorganization energy, λ, of electron transfer process.

BACKGROUND OF THE INVENTION

Electron transfer reactions are crucial steps in a variety of biological transformations ranging from photosynthesis to aerobic respiration. Studies of electron transfer reactions in both chemical and biological systems have led to the development of a large body of knowledge and a strong theoretical base, which describes the rate of electron transfer in terms of a definable set of parameters.

Electronic tunneling in proteins and other biological molecules occurs in reactions where the electronic interaction of the redox centers is relatively weak. Semiclassical theory reaction predicts that the reaction rate for electron transfer depends on the driving force ($-\Delta G°$), a nuclear reorganization parameter (λ), and the electronic-coupling strength (H_{AB}) between the reactants and products at the transition state, according to the following equation:

$$k_{ET} = (4\pi^3/h^2\lambda k_B T)^{1/2}(H_{AB})^2 \exp[(-\Delta G° + \lambda)^2/\lambda k_B T]$$

The nuclear reorginzation energy, λ, in the equation above is defined as the energy of the reactants at the equilibrium nuclear configuration of the products. There are two components of λ; "outer sphere" effects (λ_o) and "inner sphere" effects (λ_i). For electron transfer reactions in polar solvents, the dominant contribution to λ arises from the reorientation of solvent molecules in response to the change

in charge distribution of the reactants. The second component of λ comes from the changes in bond lengths and angles due to changes in the oxidation state of the donors and acceptors.

It is an object of the present invention to provide methods for the detection of target analytes exploiting changes in the solvent reorganization energy of electron transfer reactions.

SUMMARY OF THE INVENTION

In accordance with the above objects, the present invention provides methods of detecting a target analyte in a test sample. The method comprises binding an analyte to a redox active complex. The redox active complex comprises a solvent accessible transition metal complex having at least one coordination site occupied by a polar coordination group and a binding ligand which will bind the target analyte. The complex is bound to an electrode. Upon binding, a solvent inhibited transition metal complex is formed and electron transfer is detected between the solvent inhibited transition metal complex and the electrode. The methods also include applying at least a first input signal to the solvent inhibited transition metal complex.

In a further aspect, the invention provides methods of detecting a target analyte in a test sample comprising associating an analyte with a redox active complex. The redox active complex comprises a solvent inhibited transition metal complex, and a binding ligand which will bind the target analyte. Upon association, a solvent accessible transition metal complex is formed, which is then detected.

In an additional aspect, the invention provides methods of detecting a target analyte in a test sample comprising associating an analyte with a redox active complex. The complex comprises a solvent inhibited transition metal complex, a binding ligand which will bind the target analyte, and an analyte analog. The complex is bound to an electrode, and upon association, a solvent accessible transition metal complex is formed, which is then detected.

In a further aspect, the invention provides compositions comprising an electrode with a covalently attached redox active complex. The complex comprises a binding ligand and a solvent accessible redox active molecule, which has at least one, and preferably two or three coordination sites occupied by a polar coordination group, one or more of which may be a water molecule.

In a further aspect, the present invention provides an apparatus for the detection of target analytes in a test sample, comprising a test chamber comprising a first and a second measuring electrode. The first measuring electrode comprises a covalently attached redox active complex comprising a solvent accessible transition metal complex, preferably having at least three coordination sites occupied by a

polar coordination group, and a binding ligand. The apparatus further comprises an AC/DC voltage source electrically connected to the test chamber, and an optional signal processor for detection.

DETAILED DESCRIPTION

The present invention provides methods and compositions for the detection of target analytes using changes in the solvent reorganization energy of transition metal complexes upon binding of the analytes, to facilitate electron transfer between the transition metal complex and an electrode. This invention is based on the fact that a change in the oxidation state of a redox active molecule such as a transition metal ion, i.e. upon the acceptance or donation of an electron, results in a change in the charge and size of the metal ion. This change in the charge and size requires that the surrounding solvent reorganize, to varying degrees, upon this change in the oxidation state.

For the purposes of this invention, the solvent reorganization energy will be treated as the dominating component of λ. Thus, if the solvent reorganization energy is high, a change in the oxidation state will be impeded, even under otherwise favorable conditions.

In conventional methodologies using electron transfer, this solvent effect is minimized by using transition metal complexes that minimize solvent reorganization at the redox center, generally by using several large hydrophobic ligands which serve to exclude water. Thus, the ligand for the transition metal ions traditionally used are non-polar and are generally hydrophobic, frequently containing organic rings.

However, the present invention relies on the novel idea of exploiting this solvent reorganization energy to serve as the basis of an assay for target analytes. In the present invention, transition metal complexes that are solvent accessible, i.e. have at least one, and preferably more, small, polar ligands, and thus high solvent reorganization energies, are used. Thus, at initiation energies less than the solvent reorganization energy, no significant electron transfer occurs. However, upon binding of a generally large target analyte, the transition metal complexes becomes solvent inhibited, inaccessible to polar solvents generally through steric effects, which allows electron transfer at previously inoperative initiation energies.

Thus, the change in a transition metal complex from solvent accessible to solvent inhibited serves as a switch or trigger for electron transfer. This thus becomes the basis of an assay for an analyte. Closs and Miller have shown that there is a decrease in lambda in nonpolar solvents in their work on Donor(bridge)Acceptor electron transfer reactions in solution. (Closs and Miller, Science, 240, 440-447, (1988). This idea also finds conceptual basis in work done with metmyoglobin, which

contains a coordinated water molecule in the hexacoordinate heme iron site and does not undergo self-exchange very rapidly (rate constant k_{22} $1M^{-1}s^{-1}$). Upon chemical modification, the heme becomes pentacoordinate, removing the water, and the self-exchange rate constant increases significantly (rate constant k_{22} $1 X 10^4 M^{-1}s^{-1}$); see Tsukahara, J. Am. Chem. Soc. 111:2040 (1989).

Without being bound by theory, there are two general mechanisms which may be exploited in the present invention. In a preferred embodiment, the binding of a target analyte to a binding ligand which is sterically close to a solvent accessible transition metal complex causes one or more of the small, polar ligands on the solvent accessible transition metal complex to be replaced by one or more coordination atoms supplied by the target analyte, causing a decrease in the solvent reorganization energy for at least two reasons. First, the exchange of a small, polar ligand for a generally larger, nonpolar ligand that will generally exclude more water from the metal, lowering the required solvent reorganization energy (i.e. an inner sphere λ_i effect). Secondly, the proximity of a generally large target analyte to the relatively small redox active molecule will sterically exclude water within the first or second coordination sphere of the metal ion, also decreasing the solvent reorganization energy.

Alternatively, a preferred embodiment does not necessarily require the exchange of the polar ligands on the metal ion by a target analyte coordination atom. Rather, in this embodiment, the polar ligands are effectively irreversibly bound to the metal ion, and the decrease in solvent reorganization energy is obtained as a result of the exclusion of water in the first or second coordination sphere of the metal ion as a result of the binding of the target analyte; essentially the water is excluded (i.e. an outher sphere λ_o effect).

Accordingly, the present invention provides methods for the detection of target analytes. The methods generally comprise binding an analyte to a binding ligand that is either associated with (forming a redox active complex) or near to a transition metal complex. The transition metal complex is bound to an electrode generally through the use of a conductive oligomer. Upon analyte binding, the reorganization energy of the transition metal complex decreases to form a solvent inhibited transition metal complex, to allow greater electron transfer between the solvent inhibited transition metal complex and the electrode.

Accordingly, the present invention provides methods for the detection of target analytes. By "target analyte" or "analyte" or grammatical equivalents herein is meant any molecule, compound or particle to be detected. As outlined below, target analytes preferably bind to binding ligands, as is more fully described below.

Suitable analytes include organic and inorganic molecules, including biomolecules. In a preferred embodiment, the analyte may be an environmental pollutant (including pesticides, insecticides, toxins, etc.); a chemical (including solvents, polymers, organic materials, etc.); therapeutic molecules (including therapeutic and abused drugs, antibiotics, etc.); biomolecules (including hormones, cytokines, proteins, lipids, carbohydrates, cellular membrane antigens and receptors (neural, hormonal, nutrient, and cell surface receptors) or their ligands, etc); whole cells (including procaryotic (such as pathogenic bacteria) and eucaryotic cells, including mammalian tumor cells); viruses (including retroviruses, herpesviruses, adenoviruses, lentiviruses, etc.); and spores; etc. Particularly preferred analytes are environmental pollutants; nucleic acids; proteins (including enzymes, antibodies, antigens, growth factors, cytokines, etc); therapeutic and abused drugs; cells; and viruses.

By "nucleic acid" or "oligonucleotide" or grammatical equivalents herein means at least two nucleotides covalently linked together. A nucleic acid of the present invention will generally contain phosphodiester bonds, although in some cases, as outlined below, a nucleic acid analogs are included that may have alternate backbones, comprising, for example, phosphoramide (Beaucage et al., Tetrahedron 49(10):1925 (1993) and references therein; Letsinger, J. Org. Chem. 35:3800 (1970); Sprinzl et al., Eur. J. Biochem. 81:579 (1977); Letsinger et al., Nucl. Acids Res. 14:3487 (1986); Sawai et al, Chem. Lett. 805 (1984), Letsinger et al., J. Am. Chem. Soc. 110:4470 (1988); and Pauwels et al., Chemica Scripta 26:141 91986)), phosphorothioate, phosphorodithioate, O-methylphophoroamidite linkages (see Eckstein, Oligonucleotides and Analogues: A Practical Approach, Oxford University Press), and peptide nucleic acid backbones and linkages (see Egholm, J. Am. Chem. Soc. 114:1895 (1992); Meier et al., Chem. Int. Ed. Engl. 31:1008 (1992); Nielsen, Nature, 365:566 (1993); Carlsson et al., Nature 380:207 (1996), all of which are incorporated by reference). Nucleic acids containing one or more carbocyclic sugars are also included within the definition of nucleic acids (see Jenkins et al., Chem. Soc. Rev. (1995) pp169-176). These modifications of the ribose-phosphate backbone may be done to facilitate the addition of moieties, or to increase the stability and half-life of such molecules in physiological environments.

The nucleic acids may be single stranded or double stranded, as specified, or contain portions of both double stranded or single stranded sequence. The nucleic acid may be DNA, both genomic and cDNA, RNA or a hybrid, where the nucleic acid contains any combination of deoxyribo- and ribo-nucleotides, and any combination of bases, including uracil, adenine, thymine, cytosine, guanine, inosine, xathanine and hypoxathanine, etc. As used herein, the term "nucleoside" includes nucleotides, and modified nucleosides such as amino or thio modified nucleosides.

By "proteins" or grammatical equivalents herein is meant proteins, oligopeptides and peptides, and analogs, including proteins containing non-naturally occuring amino acids and amino acid analogs, and peptidomimetic structures.

As will be appreciated by those in the art, a large number of analytes may be detected using the present methods; basically, any target analyte for which a binding ligand, described below, may be made may be detected using the methods of the invention.

In a preferred embodiment, the target analyte is added or introduced to a redox active complex, which is preferably attached to an electrode. By "redox active complex" herein is meant a complex comprising at least one transition metal complex and at least one binding ligand, which, as more fully described below, may be associated in a number of different ways. By "transition metal complex" or "redox active molecule" or "electron transfer moiety" herein is meant a metal-containing compound which is capable of reversibly or semi-reversibly transfering one or more electrons. It is to be understood that electron donor and acceptor capabilities are relative; that is, a molecule which can lose an electron under certain experimental conditions will be able to accept an electron under different experimental conditions. It is to be understood that the number of possible transition metal complexes is very large, and that one skilled in the art of electron transfer compounds will be able to utilize a number of compounds in the present invention. Transition metals are those whose atoms have a partial or complete d shell of electrons. Suitable transition metals for use in the invention include, but are not limited to, cadmium (Cd), copper (Cu), cobalt (Co), palladium (Pd), zinc (Zn), iron (Fe), ruthenium (Ru), rhodium (Rh), osmium (Os), rhenium (Re), platinium (Pt), scandium (Sc), titanium (Ti), Vanadium (V), chromium (Cr), manganese (Mn), nickel (Ni), Molybdenum (Mo), technetium (Tc), tungsten (W), and iridium (Ir). That is, the first series of transition metals, the platinum metals (Ru, Rh, Pd, Os, Ir and Pt), along with Fe, Re, W, Mo and Tc, are preferred. Particularly preferred are metals that do not change the number of coordination sites upon a change in oxidation state, including ruthenium, osmium, iron, platinium and palladium, with ruthenium and iron being especially preferred. Generally, transition metals are depicted herein as M.

The transition metal ions are complexed with ligands that serve to provide the coordination atoms for the binding of the metal ion. Generally, it is the composition or characteristics of the ligands that determine whether a transition metal complex is solvent accessible. By "solvent accessible transition metal complex" or grammatical equivalents herein is meant a transition metal complex that has at least one, preferably two, and more preferably three, four or more small polar ligands. The actual number of polar ligands will depend on the coordination number (n) of the metal ion. Preferred numbers of polar ligands are (n-1) and (n-2). For example, for hexacoordinate metals, such as Fe, Ru, and Os, solvent accessible transition metal complexes preferably have one to five small polar ligands, with two to five